

Drug-induced changes of vasomotor tone in isolated human segmental pulmonary arteries

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This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. Boe, J., Simonsson, B.G. & Ståhl, E. (1980) Effect of histamine, 5-hydroxytryptamine and prostaglandins on isolated human pulmonary arteries. *Eur. J. Resp. Dis.* 61, 12-19.

II. Boe, J., Boe, M-A., Simonsson, B.G. & Ståhl, E. (1980) In vitro effects of parasympathetic agonists and atropine on human segmental pulmonary arteries. *Lung*, 157, 65-70.

III. Boe, J., Boe, M-A. & Simonsson, B.G. (1980) A dual action of histamine on isolated human pulmonary arteries. *Respiration* 40, 117-122.

IV. Boe, J. & Simonsson, B.G. (1980) Adrenergic receptors and sympathetic agents in isolated human pulmonary arteries. *Eur. J. Resp. Dis.* 61, 195-202.

V. Boe, J. & Simonsson, B.G. Effects of angiotensin II and bradykinin on isolated human pulmonary arteries. *Eur. J. Resp. Dis.*, in press.

Some additional results are also presented concerning beta₂-stimulating drugs and "slow reacting substance of anaphylaxis" (SRS-A) as well as results on the reactivity of segmental pulmonary arteries obtained at necropsy from one patient with pulmonary hypertension.



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Introduction

According to Galen who updated the views of Aristoteles (Katz 1957), the liver was the source of "natural spirits" which were distributed throughout the body via the veins. In the left ventricle, arterial blood was produced that percolated through pores of the ventricular septum. The pulmonary artery, a part of the venous system, was recognized as a nutrient vessel. The pulmonary vein had the larger responsibility of carrying air from cavities of the lung into the left ventricle.

For centuries after the discovery by William Harvey in 1628 of the real anatomical conditions of the pulmonary circulation (Harvey 1957) research on the pulmonary circulation has suffered from the handicap of the inaccessibility of the blood vessels of the lung.

The introduction of cardiac catheterization in unanaesthetized man in the 1940's opened a gateway to the systematic study of the pulmonary circulation *in vivo*. The endothelial cells of the lung vasculature are metabolically active (Ryan & Ryan 1975). Some substances are activated, others are inactivated and some are not influenced at all during their passage through the pulmonary circulation (Junod 1977). The lung is also capable of acting as an endocrine organ re-

leasing vasoactive substances in response to different stimuli (allergens, anaphylaxis, biogenic amines, peptides) (Bakhle 1975). Intravenous injection of a substance might therefore cause release of other vasoactive substances in the lung. There is also ample evidence for anastomoses between the pulmonary and the bronchial vascular system and for transport of drugs between the two systems (Daly & Hebb 1966). In order to determine *in vivo* if a drug has caused vasoconstriction or vasodilation, we need to determine at least the change in the ratio of pressure gradient across the pulmonary vascular bed (pulmonary arterial minus pulmonary venous pressure: Δp) to flow, i.e. a change in pulmonary vascular resistance (Dollery & Glazier 1966). Only studies with simultaneous measurements of cardiac output, pulmonary arterial and wedge pressures are usable for studying drug effects *in vivo* (Harris & Heath 1977a). Studies in humans of infused drugs are difficult to interpret, as there may be combined passive and active responses.

Thus epinephrine elevates left arterial pressure and increases cardiac output. This may secondarily increase transmural pressure in pulmonary vessels and result in pas-

sive pulmonary vasodilation in addition to the direct effect of epinephrine on pulmonary blood vessels (Su & Bevan 1976). Another example is 5-hydroxytryptamine, which increases intra-alveolar pressure by bronchoconstriction, this tends to occlude alveolar capillaries and thus change pulmonary hemodynamics (Rodbard & Kira 1972).

In order to collect evidence of actions on specific pulmonary vessels, it is therefore necessary to use *in vitro* techniques. Since the pioneer work of Meyer (1905) numerous studies have been performed on *in vitro* responses to various drugs of rings, strips and segments of arteries. The advantage of pharmacological studies on isolated vascular preparations is the simplicity of the response, which can be graded and closely correlated to concentrations of administered drugs.

Certain cells of the body are referred to as mediator cells. The mediator cells include a variety of morphological types such as the mast cells, basophils, platelets, enterochromaffin cells, and neutrophils. They perform their functions by the release of substances called *chemical mediators* which have a variety of biological activities (Bellanti & Kleban 1976). In allergic and probably other reactions in the airways and lung tissue, a number of chemical mediators such as histamine, "slow reacting substance of anaphylaxis" (SRS-A) or leukotrienes, 5-hydroxytryptamine, kinins, prostaglandins and acetylcholine can be released or synthesized in the tissues (Valentine 1976). There is still uncertainty as to the degree in which pulmonary vessels are involved in type I-reagin mediated antigen - antibody induced asthma, but in allergic dogs constriction of both bronchi and vasoconstriction of pulmo-

nary vessels are reported after antigen challenge (Grant et al. 1980).

Euler and Liljestrand (1946) showed alveolar hypoxia to be a powerful stimulus of vasomotor activity in pulmonary vessels, in contrast to the vasodilator effect of tissue hypoxia seen in systemic arteries. The hypoxic vasoconstriction of the pulmonary circulation reported in various animals has been attributed to release of numerous vasoactive transmitters from surrounding hypoxic tissue (histamine, angiotensin, 5-hydroxytryptamine, catecholamines, and prostaglandins), but it has not yet been possible to demonstrate that any of the above mentioned humoral transmitters are responsible for the hypoxic vasoconstriction in man (Even et al. 1975).

It will be seen that the substances suspected to induce pulmonary hypoxic vasoconstriction and the chemical mediators released in some allergic reactions are almost identical. Changes in pulmonary vasomotor tone due to chemical mediators may therefore play a part in different pathological processes in the lung and in the physiological regulation of ventilation - perfusion contributing to the efficacy of gas exchange and the maintenance of normal arterial O_2 and CO_2 tensions (Fishman 1961).

Not much is known about the effects of chemical mediators on *human* pulmonary vessels. It was therefore considered necessary to start from scratch by testing all possible chemical mediators available in a dose-response fashion on human pulmonary vessels; the choice was segmental pulmonary arteries, which were convenient to use with the present technique (Boe et al. 1975, Thulesius et al. 1978).

Aims of the study

1. To study with an in vitro technique changes in the vasomotor tone of isolated human segmental pulmonary arteries induced by chemical mediators believed to be released in allergic asthma.
2. To evaluate the contractile responses from substances thought to cause hypoxic pulmonary vasoconstriction.
3. To study the effects of the principal drugs used for the treatment of clinical asthma (adrenergics and theophyllines) on isolated human pulmonary arteries.
4. To study contractile responses with a cumulative dose-response technique allowing quantitative analysis of the pharmacologically-induced tension changes.
5. When possible characterize pharmacologically the receptors involved in the contraction and relaxation of isolated pulmonary arteries.

Material

Pulmonary artery specimens were taken from 57 patients undergoing thoracic surgery at the Department of Thoracic Surgery, Lasarettet, University of Lund. The mean age $\pm$ S.E.M. of the patients at operation was 60 ± 1.4 years, ranging from 28 to 74 years. Table 1 shows the number of patients in different decades and the sex distribution. The patient material is relatively homogenous with 82 % above 50 years and 95 % above 40 years of age. There is an excess of males (M:F=41:16) which can be explained by the fact that the majority of patients were operated for malignancies of the lung (table 2), which are more common in males in Sweden (Official Statistics of Sweden: Causes of Death 1978).

Age (years)	Number	Women	Men
20-29	1	1	0
30-39	2	1	1
40-49	7	5	2
50-59	11	1	10
60-69	28	6	22
70-79	8	2	6
Totally	57	16	41

Table 1. Age, number of patients and sex distribution

No signs of preoperative pulmonary hypertension were found in any of the patients as judged from physical examination, chest X-ray and electrocardiogram.

A majority of the patients were operated with pneumonectomy or lobar resection (table 2). These procedures allowed good access to segmental arteries of satisfactory length, whereas the preparations obtained by segmental resections were almost too short; therefore arteries from only 3 patients operated with segmental resections were tested.

One patient was operated twice, the first time with a lobar resection of the left upper lobe for a histological diagnosis of epithelial metaplasia. Half a year later he was operated once more with a lobar resection of the right lower lobe where a squamous cell carcinoma was found.

According to Brenner (1935), a transition from "elastic" to "muscular" type of artery occurs at an internal diameter of 1 mm. The segmental pulmonary arteries tested in the present study were of the "smaller elastic type" with a transsectional diameter of 2-3 mm. The artery wall consists of a media of mostly circularly oriented smooth muscle fibres sandwiched between two elastic laminae. In addition there are differing numbers of concentrically arranged laminae within the media. The number depends on the size of the artery and range from three or four in arteries of 1 mm to sixteen - twenty in arteries of 5 mm external diameter (fig

SURGICAL PROCEDURE	N	HISTOLOGICAL DIAGNOSIS	n
Pneumonectomy	22	Adenocarcinoma	14
		Alveolar cell carcinoma	1
		Large cell carcinoma	1
Lobar resection	33	Metastatic prostatae carcinoma	1
		Small cell carcinoma	1
		Squamous cell carcinoma	30
		Mycosis pulmonis	1
Segment resection	3	Epithelial metaplasia	1
		Bronchial adenoma	1
		Histiocytoma	1
		Bronchiectasis	3
Totally	58	Chron. pneumonia	1
		Tuberculosis	2
		Totally	58

Table 2. Surgical procedure and histological diagnosis. N = number of operations, n = number of specimens

I:1). A significant contractility of elastic pulmonary arteries is reported by Somlyo and Somlyo (1964). We assumed that these arteries were well-suited for screening of responses in pulmonary vascular smooth muscle, having a potentially larger effect on the circulation than "pure elastic" arteries, and because they are easier to study than smaller vessels with the testing equipment employed.

The pulmonary artery specimens were taken from macroscopically healthy lung tissue. The majority of the specimens were excised by the same surgeon (E. Ståhl). Adjacent parts of the specimens from 23

patients were used for pharmacological testing and light-microscopical investigation after fixation in basic leadacetate (Holmgren 1937) to allow examination for mast cells. Conventional examination with light-microscopy by pathologists at the Institute of Pathology, University of Lund, showed a normal anatomical appearance in all specimens investigated (I). Mast cells in the adventitia could be demonstrated by toluidine staining (Barka & Anderson 1963) in artery strips from 7 of 23 patients. It therefore seems unlikely that substances liberated from mast cells interfered to a great extent with the drugs used in our experiments.

Methods

Immediately after the excision of lung tissue, the lobar artery was identified and segmental pulmonary arteries were dissected free by the operating surgeon and at once submerged in Krebs solution at $+4^{\circ}\text{C}$. The preparation was transported to the laboratory, where further dissection of additional tissue was performed. In most cases the time between removal of lung tissue and the mounting of the first arterial specimens in the organ-baths did not exceed one hour.

To determine the diameter of the vessels, part of the artery was cut longitudinally, the circumference was measured and the transectional diameter calculated ($= \frac{\text{circumference}}{3.14}$).

Another part of the vessel was cut helically over a probe as advised by Furchgott and Bhadrakom (1953) in the way depicted by Sams and Winkelmann (1967). The vessel was tied at one end by a piece of 0.4 silk; a pointed stainless steel obturator, slightly smaller than the lumen, was inserted into the untied end of the vessel and pushed through its wall just proximal to the tie. Both ends of the obturator were anchored in a cork board. One blade of a pair of microdissecting scissors was inserted into the lumen, and the vessel was cut open in a spiral fashion by a

gentle pull on the silk tie at a 45° angle while the cut was made. The spirally cut vessel was then fixed at both ends on a cork board and small segments of 13 mm length and 2–3 mm width were cut from the arterial strip with a scalpel and by the aid of a standard.

The vascular strips were stored in a refrigerator at $+4^{\circ}\text{C}$ until the testing, which normally took place on the same day. In some instances the testing procedure continued on the following morning.

Helically cut arterial strips were mounted vertically by Hemoclips[®] at the upper end, with a screw-attachment at the base (fig. 1:2). The preparation could be stretched at the lower fixation point with the aid of a micrometer.

A preload of 0.5–1.0 g was added by altering the micrometer in order to suspend the preparation so that it remained straight when immersed in the bath (IV). The chosen preload corresponds well with the "zero load length" of 1 g, expressed as minimum load required to remove any visible slack in the vessel strip when mounted in Krebs solution, found by Banks et al. (1978) in their tension-balance studies on post-mortem isolated human pulmonary arteries.

The specimen was submerged in an organ-

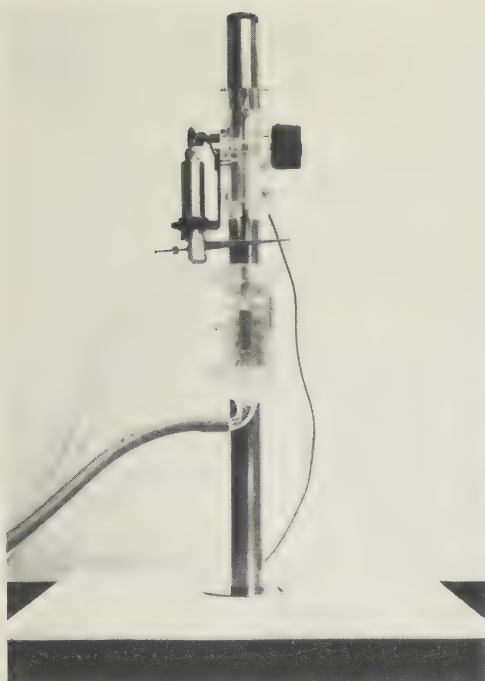


Fig. 1. Organ-bath with a Statham Model UL 5 force-transducer at the top left for recording muscle tension.

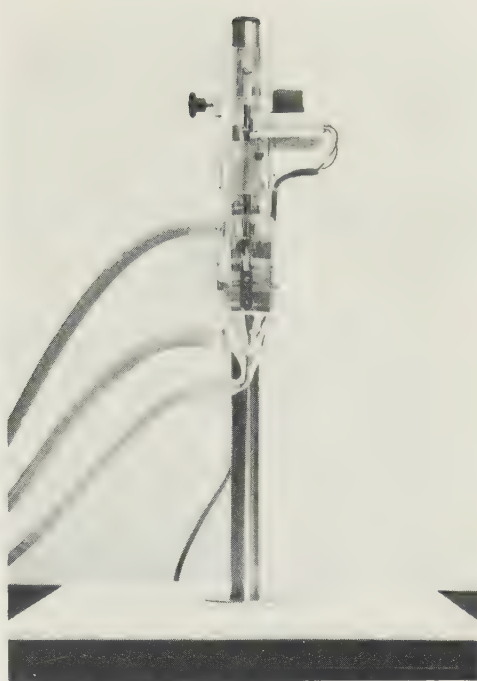


Fig. 2. Organ-bath with a Swema SG 4-45 force-transducer at the top right for recording muscle tension.

bath containing 20 ml normal Krebs solution composed according to Gant and Dyer (1971). It was prepared by the Pharmacy of the University Hospital in Lund, and had the following composition (mM): NaCl 115.33, KCl 4.69, CaCl_2 2.45, MgSO_4 1.14, Na_2

(EDTA) 0.027, KH_2PO_4 1.18, NaHCO_3 22.14, Glucos 7.42, pH=7.4.

The double walled organ-bath, made by a skilled glass-blower, was perfused with water held at a constant temperature of $+37^\circ\text{C}$ by a water-jacket. It was bubbled continu-

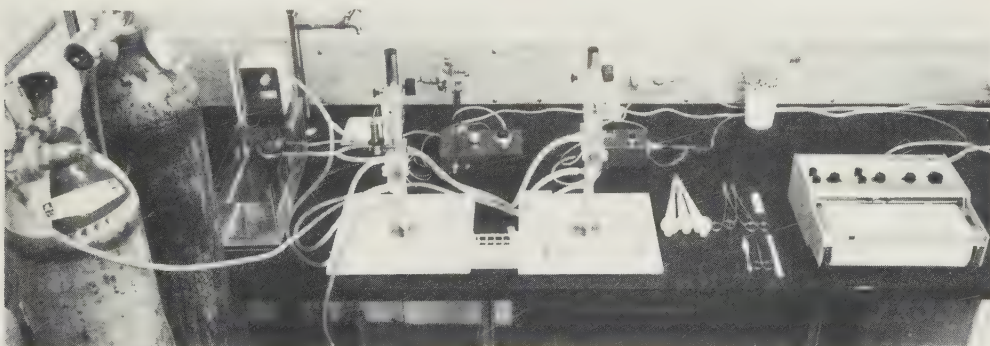


Fig. 3. Testing equipment consisting of a two-channel W+W₆₀₀ recorder to the right, two organ-baths with transducers and potentiometers in the middle and a water-jacket and two gastubes to the left. A number of Eppendorf® micropipettes are lying between the one organ-bath and the recorder.

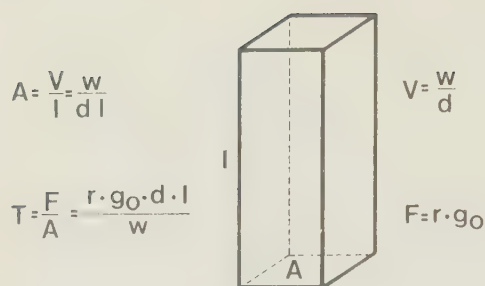


Fig. 4. Principle of calculation of wall-tension.

A =cross-sectional area, V =volume (m^3),

l =length (m), w =weight (kg), d =density.

T =tension (N/m^2), F =force (N).

r =response (kg), g_0 =gravitational constant.

ously with a mixture of 95 % O_2 and 5 % CO_2 to ensure a good oxygenation of the arterial strips, creating aerobic conditions even in deeper layers (Furchgott 1960).

Initially we had to use a single organ-bath; later two identical organ-baths could be operated simultaneously. Isometric tension changes were recorded with a Statham Model UL 5 force-transducer (fig. 1) in the one organ-bath, and a Swema SG 4-45 force-transducer (fig. 2) in the second. The tensions obtained with the two strain-gauges were similar and were continuously recorded on a W+W₆₀₀ two-channel recorder. The testing equipment is shown in fig 3.

When the pulmonary arterial specimens were mounted, the ends of the preparations were inevitably crushed; the actual working length of the preparations (as measured between impressions of the strips after dismounting) was estimated to be 10 mm. After dismounting of the strip, the crushed ends were trimmed off and the remaining preparation weighed (wet weight). As Joiner et al. (1975a) reported poor in vitro responsiveness of pulmonary vessels being stretched during dissection, great care was taken during all handling of the preparations to avoid stretching.

We tested 174 pulmonary arterial specimens with a mean weight $\pm$ S.E.M. of 21.3 $\pm$ 0.3 mg, ranging from 12 to 28 mg.

A comparison of tension changes in dif-

ferent preparations is possible when related to transsectional area (A) of the preparation. (A) was calculated by entering the wet weight (w) of the specimen together with the actual working length (l) into the formula

$$A = \frac{w}{l \cdot d},$$

assuming a tissue density (d) of 1.06 (Mc Donald 1974, Keatinge 1968). The force was initially measured in grammes and calculated in Newton (N). The principle for calculation of the wall-tension into units per transsectional area in N per m^2 is shown in fig 4. The method of calculation is similar to that used in previous studies on isolated human pulmonary arteries (Banks et al. 1978) and human bronchi (Boe et al. 1975, Thulesius et al. 1978).

The vascular strips were allowed to equilibrate for 1–2 hours in the organ-bath to obtain a stable condition before measurements were done. When repeated testings were performed, the specimens were washed several times with fresh buffer solution until relaxation to the initial base-line tension was obtained, a procedure that usually required 10–20 minutes. A new testing procedure was never started until a stable condition was obtained, which usually required at least an additional 30 minutes. When an arterial strip was tested for contractility with more than one substance, the order of drug administration was at random.

The cumulative dose technique of van

Rossum (1963) was adopted to obtain a dose-response curve. The drug was added stepwise in increasing concentrations until the contraction no longer changed with further increase of drug. After each addition of drug, time was allowed for the strip to reach equilibrium (the maximal response reached with that particular concentration) before the concentration of the agonist was increased. No fixed interval therefore existed for the duration of exposure to a particular concentration of the agonist. Threshold concentration for any given agent was defined as the concentration producing a detectable deflection of $4.6 \cdot 10^{-3}$ g on the W+W₆₀₀ recorder.

Drugs were administered into the organ-bath with the aid of Eppendorf comfortips[®] micropipettes with disposable tips at volumes of 5–50 μ l.

The following drugs were used: histamine hydrochloride (Apoteksbolaget, I), prostaglandin F₂ α (Astra, I, II, IV, V), prostaglandin E₁ (Sigma, I), 5-hydroxytryptamine oxalate (Sigma, I, II, IV), clemastine fumarate (Sandoz, I), histamine chloride (Apoteksbolaget, II, III, IV, V), indomethacin (Dumex, I), propranolol hydrochloride (ICI,

I, IV), atropine sulfate (ACO, I, II), methysergide maleate (Sandoz, I), phenoxybenzamine hydrochloride (SKF, I, IV), indoramine hydrochloride (Wyeth, I, IV), polyphlorelin phosphate (LEO, I), acetylcholine chloride (Roche, II, IV), carbachol chloride (ACO, II, IV), norepinephrine bitartrate (Apoteksbolaget, II, IV, V), mepyramine maleate (Sandoz, III), cimetidine (SKF, III), epinephrine bitartrate (ACO, IV), isoprotenerol sulfate (Apoteksbolaget, IV), salbutamol sulfate (Allan & Hamburgs, IV), terbutaline sulfate (Draco, IV), theophylline (ACO, IV), angiotensin II (CIBA, IV), bradykinin triacetate (Sigma, V), (Sar¹, Ile⁸)-angiotensin II (Beckman, V). Given values of concentrations are valid for the active substance, and all drugs are compared in molarity (log mol/l) except polyphlorelin phosphate (which consists of a mixture of polymers of phlorelin, therefore mol weight is missing) and "slow reacting substance of anaphylaxis" (SRS-A).

Conventional statistical methods were employed. Student's t-tests were used when comparing groups with respect to metric variables and levels of significance refer to two-tailed test.

Results

Thirty-six patients smoked at the time of operation, all of them 10 cigarettes or more a day and/or at least 50 g pipe tobacco weekly. Eleven patients were ex-smokers, i.e. they had ceased smoking at least one month before operation; ten patients were non-smokers. No statistically significant difference

could be demonstrated between the contractions induced with prostaglandin $F_{2\alpha}$ or histamine in preparations taken from the three smoking categories (table 3). It therefore seems unlikely that smoking habits influenced tension changes of the isolated pulmonary arteries.

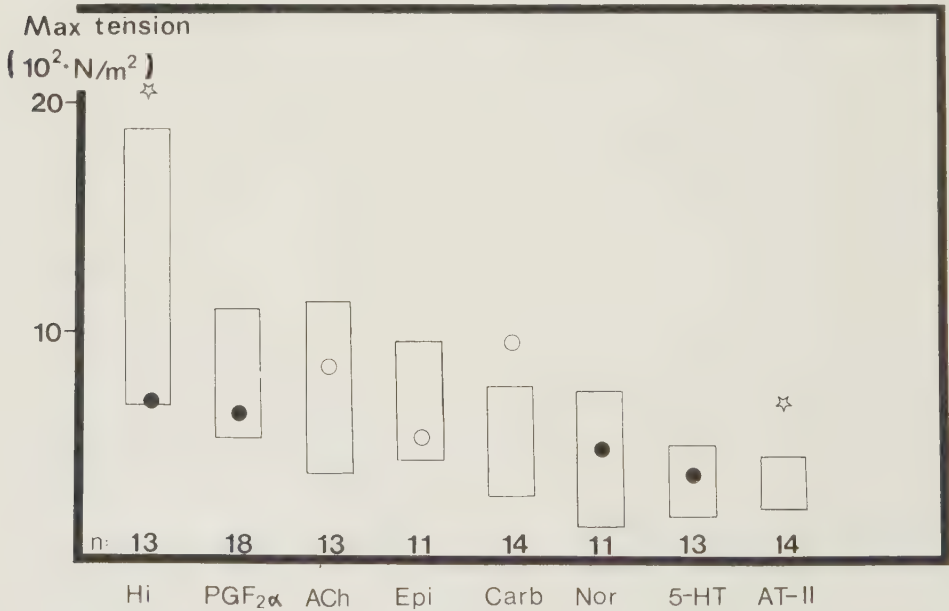


Fig. 5. Maximal contractory responses (N/m^2) of three patients less than forty years of age (☆=28, ●=34 and ○=36 years) in comparison to the 95 % confidence intervals (□) of the mean values of the test substances. n=number of specimens. Hi=histamine, PGF_{2α}=prostaglandin $F_{2\alpha}$, ACh=acetylcholine, Epi=epinephrine, Carb=carbachol, Nor=norepinephrine, 5-HT=5-hydroxytryptamine, AT-II=angiotensin II.

Information on preoperative airways obstruction judged from $FEV_{1.0}$ (forced expiratory volume in one second) was available in all except one patient. Fourteen patients had airways obstruction, 10 of them were smokers, 2 ex-smokers and 2 non-smokers. There was no statistically significant difference between the contractions induced with histamine or prostaglandin $F_{2\alpha}$ (table 3) in obstructive and normal subjects.

Fig. 5 shows that the responses induced by histamine and angiotensin II in a patient below 30 years are greater than the 95 % confidence intervals of the means. In patients between 30 and 40 years a value greater than the above stated confidence interval was found in only one experiment. If a reliable comparison of tension differences due to age were to be done, more young patients would have to be investigated. This is difficult since extremely few patients are operated with excision of lung tissue before 30 years of age.

a) Resting tone

After mounting and equilibration for more than one hour, it was not possible to show a relaxation of the arterial specimens from the resting steady state after exposure to adrenergic agonists or antagonists (I, IV), theophyllines (IV), parasympathetic agonists and atropine (II), antihistaminics: nonspecific (I) or H_1 - and H_2 -receptor antagonists (III), antiserotonin agents (I), indomethacin (I), polyphlorethin phosphate (I), prostaglandin E_1 (I) or the peptides angiotensin II and bradykinin (V). This indicates that the isolated human segmental pulmonary arteries have no "active" resting tone, which is contrary to findings in isolated human bronchial preparations where some basal tone has been demonstrated (Simonsson et al. 1972, Simonsson et al. 1973, Thulesius et al. 1978).

	n _i		Max. tension				ED 50				Threshold dose	
			$10^2 \cdot N/m^2$		log mol/l		log mol/l		log mol/l		log mol/l	
	PGF ₂ α	Hi	PGF ₂ α	Hi	PGF ₂ α	Hi	PGF ₂ α	Hi	PGF ₂ α	Hi	PGF ₂ α	Hi
Smokers	13	8	7.8±2.15	16.2±4.06	-3.98±0.1	-4.13±0.26	5.68±0.14	-5.05±0.32	-7.9±0.14	-7.64±0.5		
Ex-/nonsmokers	5	7	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
			6.1±4.7	12.7±4.2	-3.97±0.1	-3.86±0.43	-5.15±0.15	-5.77±0.31	-7.18±0.46	-7.69±0.34		
Obstructive	4	3	6.7±2.6	18.6±7.6	-4.06±0.25	-3.54±0.1	-5.55±0.14	-5.32±0.44	-8.17±0.28	-7.67±0.6		
Normal subjects	14	12	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
			7.6±1.0	12.9±2.85	-3.87±0.13	-4.1±0.27	-5.54±0.18	-5.41±0.26	-7.78±0.21	-7.72±0.34		

Table 3. Comparison of mean max. tension (N/m^2) at average max. concentration, ED 50 and threshold doses (log mol/l) $\pm$ S.E.M. for smokers and ex-/nonsmokers, and for obstructive and normal subjects. PGF $_{2\alpha}$ = prostaglandin $F_{2\alpha}$, Hi = histamine, ni = number of patients, n.s. = non significant. Statistical method = t-test for two means.

No spontaneous contractile activity was seen in the pulmonary arteries which corresponds with results reported by Mikkelsen (1979a) from pulmonary vascular specimens from 6 patients. On the other hand, he reported spontaneous contractile activity in isolated human pulmonary veins. Quantitative analysis of pharmacologically-induced tension changes in arteries with spontaneous rhythmic contractility (such as isolated human uterine arteries (Cuthcin et al. 1964)), is extremely difficult. Absence of spontaneous contractility seems to be a prerequisite for such analysis.

b) Contractility

Contractile responses induced with angiotensin II (V), prostaglandin $F_2\alpha$ (I, II, IV, V), 5-hydroxytryptamine (I, II, IV, V), norepinephrine (II, IV, V), histamine (I, II, III, IV, V), acetylcholine (II, IV), carbachol (II, IV) and epinephrine (IV) were reproducible for at least 8 hours at $+37^\circ\text{C}$, and for 24–30 hours after storage at $+4^\circ\text{C}$ in a refrigerator. This corresponds well with the results of Mikkelsen (1979a), who reported reproducible contractile responses of isolated human pulmonary arteries to norepinephrine and potassium for 12 hours at $+37^\circ\text{C}$ and after 24 hours in refrigerator ($+4^\circ\text{C}$). Mikkelsen et al. (1978a, 1979b) also reported reproducible pharmacologically-induced contractions of isolated human peripheral veins and mesenteric arteries and veins for 12 hours at $+37^\circ\text{C}$ and for 24 hours at $+4^\circ\text{C}$. and Thulesius and Gjörös (1974) showed reproducible contractions in isolated vessels from normals and patients with varicose veins after 5–6 hours incubation at $+4^\circ\text{C}$. Isolated human basilar and anterior cerebral arteries taken at necropsy within one hour of death showed no change in reactivity after 20 hours when kept in the testing chamber (Al-

len et al. 1976). As early as 1905, Meyer showed that arterial preparations stored for 13 days in $+2$ – 3°C could be stimulated to contraction, and reproducible responses to electrical stimuli were obtainable for 3–4 hours in the organ-bath (Meyer 1905). Starling et al. (1975) demonstrated reactivity of isolated human basilar arteries to drugs at least 90 hours after death..

Pharmacological stimulation of *bronchial* specimens obtained *post mortem* and stored in Krebs solution ($+3^\circ\text{C}$) elicited contractions up to 6–10 hours after removal from the body. Repeated pharmacological testing showed reproducible results for as long as ten hours with the bronchial preparation in the organ-bath at $+37^\circ\text{C}$ (Thulesius et al. 1978), and 12–16 hours for the isolated post-mortem human basilar arteries (Starling et al. 1975). Human vascular muscle similar to bronchial smooth muscle preparations thus seem to be well suited for in vitro testing; reproducible contractions can be obtained.

Cumulative dose-response curves in the form of contraction of the pulmonary vascular strips were obtained with histamine (I, III), prostaglandin $F_2\alpha$ (I), 5-hydroxytryptamine (I), acetylcholine (II), carbachol (II), norepinephrine (IV), epinephrine (IV), angiotensin II (V) and SRS-A. The maximal contractile response is defined as the maximal tension achieved by supramaximal doses of the agonist tested. Examples on cumulative dose-response curves are shown in fig. I:3 (prostaglandin $F_2\alpha$), fig. II:1 (acetylcholine), fig. III:2 (histamine), fig. IV:1 (norepinephrine), fig. IV:3 (epinephrine) and fig. V:1 (angiotensin II).

''Slow-reacting substance of anaphylaxis'' (SRS-A) is held responsible for the greater part of the antihistamine-resistant bronchoconstriction in asthma (Brocklehurst 1976). It induces a marked long-lasting con-

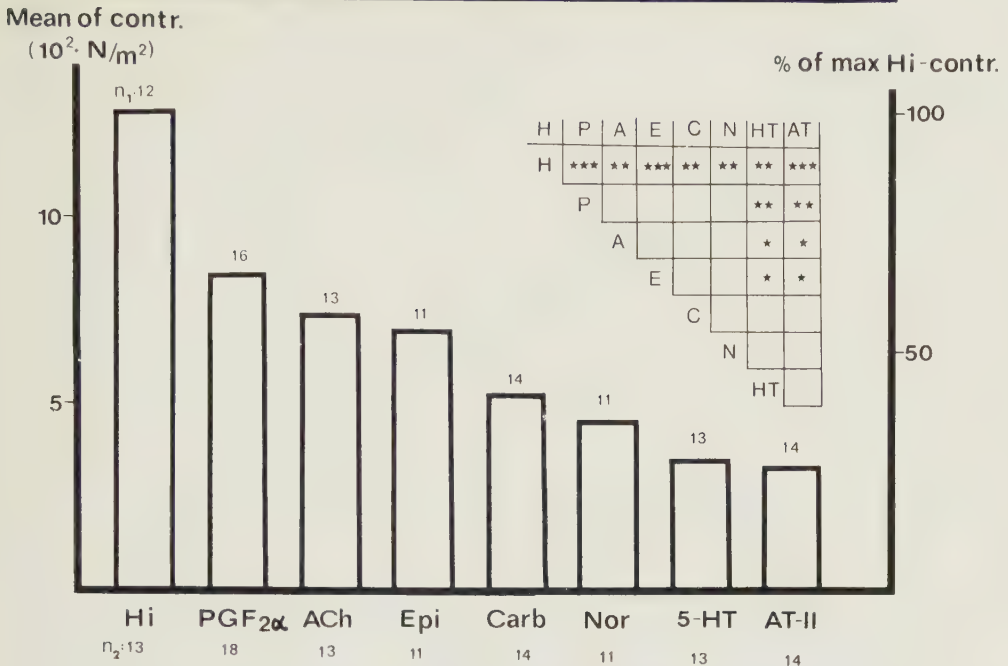


Fig. 6. Mean max. contraction (N/m^2) and percentage (%) of average histamine-induced contraction.

n_1 =number of patients, n_2 =number of pulmonary artery specimens. Inserted the results of statistical analysis (student t-test) for the contractory responses. ★= $p < 0.05$, ★★= $p < 0.01$, ★★★= $p < 0.001$, open squares=non significant.

Hi (H)=histamine, PGF₂α (P)=prostaglandin F₂α, ACh (A)=acetylcholine, Epi (E)=epinephrine, Carb (C)=carbachol, Nor (N)=norepinephrine, 5-HT (HT)=5-hydroxytryptamine, AT II (AT)=angiotensin II.

striction of isolated human bronchi (Brocklehurst 1970, Mathé & Strandberg 1971). In this study SRS-A was generated by a histamine liberation-procedure on cat paws by compound 48/80 and was purified by solvent extraction, silic acid and anion exchange chromatography as previously described (Strandberg & Uvnäs 1971). One unit (U) of SRS-A has been defined as the threshold dose to SRS-A for a contractile action on the isolated guinea-pig ileum (Strandberg 1971). SRS-A was tested on segmental pulmonary artery strips from a 72 years old man operated with lobectomy for squamous cell carcinoma with 2.5 U/ml to 300 U/ml bath fluid; a cumulative dose-response contraction curve could be elicited

with a threshold dose of 40 U/ml, median effective dose (ED 50) of 79 U/ml and a concentration of 155 U/ml giving rise to maximal contraction. The maximal contraction was $3.35 \cdot 10^2 \text{ N/m}^2$. When no more contraction could be induced by higher concentrations of SRS-A, prostaglandin F₂α in doses -4.45 to $-3.85 \log \text{ mol/l}$ was added to the bath. A vigorous contraction of $3.47 \cdot 10^3 \text{ N/m}^2$ followed. This indicates that the contractions of the isolated pulmonary arteries due to SRS-A are of small magnitude compared to those inducible by prostaglandins. Relatively high activity of SRS-A was necessary to induce a contraction; this correlates well with earlier results on isolated bronchial smooth muscle (Mathé & Strand-

	Hi	PGF ₂ α	ACh	Carb	Epi	Nor	5-HT	AT-II
Hi								
PGF ₂ α					**	*		**
ACh					*			*
Carb					**	**		**
Epi	***	***	***	**				
Nor	***	***	***	*				
5-HT	*	*						
AT-II	***	***	***	***	*	**	***	

ED 50

THRESHOLD DOSE

Table 4. Results of statistical analysis (student t-test for two means) concerning median effective dose (ED 50) and threshold doses between the contractory substances histamine (Hi), prostaglandin F₂α (PGF₂α), acetylcholine (ACh), carbachol (Carb), epinephrine (Epi), norepinephrine (Nor), 5-hydroxytryptamine (5-HT) and angiotensin II (AT-II). *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, open squares = non significant.

berg 1971, Strandberg & Uvnäs 1971, Strandberg & Hedquist 1975). Because of great difficulties in obtaining SRS-A it was not possible to test strips from more patients. Great caution is necessary in the estimation of the results from a single patient, but this study indicates that SRS-A, at least in higher doses, can contract isolated human pulmonary arteries.

The chemical structure of SRS-A from various sources is currently proposed to belong to a novel group of compounds, the leukotrienes. These are formed from arachidonic acid by a pathway initially involving formation of a 5-hydroxy-peroxy-derivate (Samuelsson et al. 1980). SRS-A is presumed to consist of leukotrienes LTC₄ and LTD₄, both potent constrictors of isolated human bronchi (Hedquist et al. 1980). Dahlén and

Hedquist (1980) found LTC₄ and LTD₄ about 2000 times as potent as histamine and 500 times as potent as prostaglandin F₂α on isolated human bronchi, making them the most potent bronchoconstricting substances known today.

Fig. 6 shows the mean maximal contractions and the results of statistical analysis (student's t-tests for two means) for the contractory substances tested. For comparison the mean maximal contraction induced with *histamine* (I), which gives rise to the strongest contraction, is chosen as 100 per cent. The mean maximal contractions of the other contractory substances are then calculated in per cent of the histamine-induced contraction. The mean maximal contraction of prostaglandin F₂α, acetylcholine and epinephrine corresponds to more than 50 %,

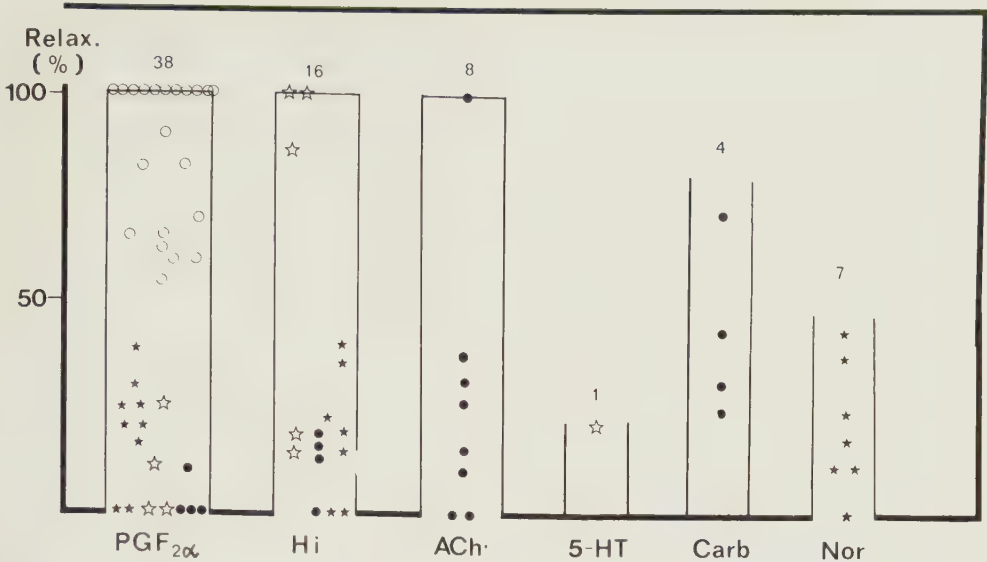


Fig. 7. Relaxing potency of isoproterenol (●), prostaglandin E₁ (○), bradykinin (★), and theophylline (☆) on isolated human pulmonary arteries.

Dots (●, ○) or stars (★, ☆) indicate a single experiment on a pulmonary artery strip. At the top of the columns numbers of specimens tested. The contractory substances stated at the abscissa were given in supramaximal doses to induce maximal contraction. PGF₂α=prostaglandin F₂α, Hi=histamine, ACh=acetylcholine, 5-HT=5-hydroxytryptamine, Carb=carbachol, Nor=norepinephrine.

whereas that of 5-hydroxytryptamine and angiotensin II is about 25 % of the mean maximal contraction induced by histamine.

Histamine caused a contraction significantly stronger than that from the other substances tested; prostaglandin F₂α, acetylcholine and epinephrine in turn induced contractions significantly greater than that of 5-hydroxytryptamine and angiotensin II (fig. 6). No statistically significant differences were found between the mean maximal contractions of acetylcholine and carbachol (table II:4, fig. 6) or between norepinephrine and epinephrine respectively (table IV:2, fig. 6).

Significant differences between threshold doses (log mol/l) or median effective dose (ED 50 in log mol/l) for the test substances are shown in table 4. No statistically

significant differences were found between histamine, prostaglandin F₂α, acetylcholine or carbachol. The actual mean threshold and ED 50 doses and ranges in log mol/l as well as ranges of contraction in N/m² for the contractory substances are given in the respective papers.

c) Relaxation

Drugs which cause relaxation cannot be tested on untreated pulmonary artery strips, since such strips possess no resting tone and are therefore already in a relaxed state. For quantitative studies of the relaxing effects of drugs, the strips must first be contracted. The relaxing effect of drugs was therefore tested after maximal contraction with supramaximal doses of constricting substances. The degree of relaxation was calcu-

CONTRACTORY SUBSTANCES	SALBUTAMOL Sulfate (-8.92 to -3.6)		TERBUTALIN Sulfate (-9.25 to -3.92)	
	n_1	n_2	n_1	n_2
HISTAMINE CHLORIDE (-8.9 to -3.57)	4	4	4	5
CARBACHOL CHLORIDE (-9.46 to -3.92)	2	2		
ACETYLCHOLINE CHLORIDE (-8.86 to -3.53)	1	1	0	0
PROSTAGLANDIN $F_{2\alpha}$ (-9.15 to -3.82)	6	6	8	8
NOREPINEPHRINE BITARTRATE (-8.83 to -3.51)			1	1
TOTALLY	13	13	13	14

Table 5. Number of experiments with the selective β_2 -stimulants terbutaline and salbutamol. Doses in log mol/l in brackets. n_1 = number of patients, n_2 = number of segmental arterial preparations.

lated in per cent of the maximal possible relaxation, i.e. a return of the tension to the base-line from a maximal contraction.

Isoproterenol caused some degree of relaxation in 14 of 20 tested arterial strips, but only one specimen showed total reversibility (100 % relaxation (fig. IV:4)).

Theophylline relaxed 8 of 10 specimens to varying degrees, a total reversal occurred in two specimens. There was no statistically significant difference between the relaxation obtained with isoproterenol and theophylline.

Bradykinin caused some relaxation in 18 of twenty-three pulmonary artery specimens, but none was totally relaxed. The maximal relaxation produced was 43 per cent (fig. V:4).

Prostaglandin E_1 totally relaxed 11 of 21

pulmonary artery strips contracted with prostaglandin $F_{2\alpha}$ (I). All specimens reacted and the smallest induced relaxation was 55 % (fig. 7).

Acetylcholine or carbachol could not relax 11 specimens after contraction with norepinephrine, prostaglandin $F_{2\alpha}$, histamine or 5-hydroxytryptamine (II). On the contrary, in some of the specimens addition of acetylcholine or carbachol induced a further contraction.

Somewhat unexpectedly the "selective" β_2 -agonists salbutamol or terbutaline failed to relax 13 and 14 artery strips contracted by histamine, carbachol, acetylcholine, prostaglandin $F_{2\alpha}$ and norepinephrine respectively (table 5.). This is in agreement, however, with a recent report that terbutaline given orally or subcutaneously to a patient

with primary pulmonary hypertension was without effect on the vascular resistance (Person & Proctor 1979).

The relaxing potency for each tested contractory substance is expressed in per cent of reversal of the maximal contraction (fig. 7). Relaxation of more than 50 % was obtainable in only five specimens contracted with histamine, acetylcholine or carbachol with isoproterenol, bradykinin or theophylline. For prostaglandin $F_{2\alpha}$ -induced contraction, a maximal relaxation of only 39 % could be obtained with any of the afore-mentioned drugs. The relaxing effect of isoproterenol, theophylline and bradykinin on isolated human pulmonary arteries is thus not very impressive. The relaxation on prostaglandin $F_{2\alpha}$ -contracted vessels by prostaglandin E_1 , however, showed a mean relaxation ($\pm$ S.E.M.) of 85.5 ± 3.8 % which is significantly greater ($p < 0.001$) than the relaxation induced by bradykinin (19.4 ± 6.5 %).

d) Receptors

The contractory effect of histamine was antagonized by clemastine fumarate, an unspecific antihistaminic drug (I). A competitive antagonism could be demonstrated with the aid of the specific H_1 -antagonist mepyramine (fig. III:3). The histamine H_2 -antagonist cimetidine (Brogden et al. 1978) given prior to histamine caused a slight potentiation of the histamine-induced contraction (fig. III:3). A similar potentiation was reported with burimamide (Goadby & Phillips 1973) in isolated perfused guinea-pigs. Cimetidine, too, abolished the relaxation of H_1 -blockers (mepyramine) on histamine-contracted isolated vessels (III). On the basis of these findings, we postulate the presence of two different histamine-receptors: H_1 - and H_2 -receptors in human segmental pulmonary arteries. A difficulty in

the study of histamine-receptors in the pulmonary vasculature is that the distribution of H_1 - and H_2 -receptors may vary between different areas of the same vessels, thus Grimm et al. (1978) reported that histamine decreased the fraction of total pressure drop occurring upstream from the midpoint while it increased it downstream in canine lung lobe vasculature. This might be due to different types of histamine-receptors on the arterial and venous sides in at least the canine pulmonary circulation. The presence of two histamine-receptors has previously been suggested in the pulmonary circulation of isolated guinea-pig lungs (Okpako 1972, Goadby & Phillips 1973, Türker 1973), in anaesthetized dogs (Tucker et al. 1975) and in isolated neonatal and adult canine pulmonary arteries (Newman et al. 1979), as well as in isolated extra- and intracranial arteries of the cat (Edvinsson & Owman 1975), in gastrointestinal circulation of anaesthetized dog (Charbon et al. 1980) and in the airways of guinea-pigs (Dulabh et al. 1980) and man (Eiser et al. 1979).

Adrenergic alpha- and beta-receptors have been demonstrated with in vitro technique in human coronary arteries (Andersson et al. 1972). The movement of the norepinephrine and epinephrine dose-response contraction curves to the right with the alpha-receptor blocking substances phenoxybenzamine or indoramine indicates competitive antagonism at specific adrenergic alpha-receptors in human pulmonary arteries (IV). These results correlate well with the findings of Joiner et al. (1975a), who reported an inhibition of the contractile response to lower levels of norepinephrine in three experiments with the alpha-adrenergic blocker phentolamine (10^{-8} M), whereas responses induced by 10^{-4} M norepinephrine were lowered only slowly or not at all. The presence of beta $_1$ -receptors

can be suggested, but it was not possible to demonstrate β_2 -receptors with the aid of the specific β_2 -stimulating drugs salbutamol or terbutaline (IV).

Acetylcholine and carbachol caused contraction of the isolated pulmonary arteries (I, Boe et al. 1978). This action was inhibited by atropine, a blocker of the muscarinic effects of acetylcholine. These observations agree with those of Joiner et al. (1975a), who showed an inhibition by atropine (10^{-8} M) of the contractile responses to lower levels of acetylcholine, whereas responses induced by 10^{-4} M acetylcholine were either slightly depressed or not affected. The latter results indicate a competitive antagonism of atropine. The existence of cholinergic muscarinic receptors in human pulmonary segmental arteries is suggested on the basis of the results stated above.

The contractile responses to 5-hydroxytryptamine were blocked by an antiserotonin drug, methysergide; the existence of serotonergic receptors in the pulmonary artery strips might thus be assumed (I). This assumption is shared by Gyermek (1966) who postulated an antagonistic effect for methysergide on smooth muscle 5-hydroxytryptamine-receptors.

A competitive antagonism of angiotensin II with (Sar¹Ile⁸-)-angiotensin II, an angiotensin II-antagonist (Türker et al. 1972) indicates the existence of angiotensin II-receptors in pulmonary arteries (V).

The contractory effect of prostaglandin $F_{2\alpha}$ and the relaxation induced with prostaglandin E_1 could not be blocked by an antihistaminic (clemastine) or an antiserotonin drug (methysergide), α - (phenoxybenzamine or indoramine) or β -adrenergic blockers (propranolol), a non-steroid anti-phlogistic (indomethacin), an antimuscarinic (atropine) or polyphlorethin phosphate (I).

This indicates the presence of prostaglandin $F_{2\alpha}$ - and E_1 -receptors in human pulmonary arteries.

e) Tension changes of isolated post mortem specimens from a patient with pulmonary hypertension.

Human bronchi taken post mortem are suitable for pharmacological in vitro tests if performed no later than 10 hours after death (Boe et al. 1975, Thulesius et al. 1978). In order to compare reactions to drugs in normal pulmonary vessels and pathologically changed lung vessels, we tried to obtain specimens from patients dying from pulmonary hypertension. During a period of about 3 years, however, it was only possible to obtain pulmonary vessels from one patient, a 38 year old man dying from primary pulmonary hypertension. Histologically his pulmonary arteries showed a marked thickening of the arterial walls with proliferation of the intima and duplication of the elastica. In the pulmonary vessels there were numerous fibrotic plaques. Segmental pulmonary artery specimens were removed from the body 9 1/2 hour after death and immediately tested. Contractions of the artery specimens were obtained (table 6) with prostaglandin $F_{2\alpha}$ (doses: -8.45 to -3.13 log mol/l) and histamine (doses: -8.65 to -3.32 log mol/l) with a max. response of $3.26 \cdot 10^2$ N/m² and $4.83 \cdot 10^2$ N/m² respectively.

The pathologically changed arteries still had the ability to contract in response to challenge with substances normally occurring in the body, although the contractions induced were of small magnitude. This indicates that even in patients dying from pulmonary hypertension, an active vasoconstriction of the pulmonary arteries may be of importance.

The contraction induced by prostaglandin

	Maximal tension				ED 50		Threshold dose	
	$10^2 \cdot \text{N/m}^2$	Hi	$\text{PGF}_2\alpha$	Hi	$\text{PGF}_2\alpha$	Hi	$\text{PGF}_2\alpha$	Hi
Post mortem specimens	3.26	4.83	-4.29	-4.64	-4.81	-5.48	-7.85	-8.0
Thoracotomy specimens	8.17 (1.21-23.2)	12.86 (0.92-32.8)	-3.97 (4.8-3.3)	-3.91 (6-3.32)	-5.56 (6.69-4.5)	-5.53 (7-4)	-7.71 (8.45-5.8)	7.78 (9.65-5.48)

Table 6. Comparison of tension changes of post mortem specimens from a patient with pulmonary hypertension and average values for specimens obtained at thoracotomy. Hi = histamine. $\text{PGF}_2\alpha$ = prostaglandin $\text{F}_{2\alpha}$ Ranges in brackets.

$\text{F}_{2\alpha}$ could be totally reversed by prostaglandin E_1 in the dose -5.45 to -3.13 log mol/l, which indicates that prostaglandin E_1 might be of value in treatment of pulmonary hypertension. Clinical experience with this substance is, however, very limited (I, Fein & Frishman 1980).

General Discussion

a) General aim

An estimation of the effects of known chemical mediators on human segmental pulmonary arteries was undertaken as a basis for possible further studies of pathophysiological effects on pulmonary vessels in clinical situations such as asthma, hypoxia and pulmonary hypertension. Great care must be taken in the interpretation of differences in contractility to different agents. Even if the testing procedure in all experiments is identical, a comparison of reactions on preparations from different subjects must be evaluated with great caution. Nevertheless, the results from the comparison indicates differences in maximal efficacy, median effective dose and potency between the different substances tested.

Noticeable is the great span of maximal contractions, threshold doses and ED 50's (I, II, IV, V). This reflects marked differences in the responses of preparations from different patients, pointing to a great inter-individual difference in sensitivity of the smooth muscle, a phenomenon also reported in isolated human (Simonsson et al. 1973, Boe et al. 1975, Thulesius et al., 1978) and animal bronchial preparations (Niewoehner et al. 1979). When specimens from the *same* pa-

tient were tested with two different agents there was not such a great span in the responses. Thus there was no significant difference between results from simultaneous norepinephrine and epinephrine challenges on adjacent preparations in eleven patients (table IV:2). A difference in the relative concentrations of receptors in the same organ between different individuals has been postulated by Furchgott (1976) for adrenergic receptors. Nickerson and Kunos (1977) pointed to an interconversion of adrenoreceptors as a possible mechanism for adaption of the response of an organ or tissue to its metabolic state. To what extent the above-mentioned mechanisms may explain the interindividual differences in response reported in this study is unknown.

b) Animal versus human tissues

Because of the easier accessibility of isolated vessels from animals compared to the much harder to obtain human tissue for studies of drug responses, isolated blood vessels from animals have usually been employed even to predict mechanisms of action in man. There is, however, a variation in reactivity both between different arteries of the same species (Bohr et al. 1961, table 7) and a

Table 7. In vitro investigations on isolated human arteries
 n_1 = number of patients
 n_2 = number of specimens tested

SUBSTANCES	n_1	n_2	Type of arteries	Diameter of vessel (mm)	RESPONSE	REFERENCE
Serotonin, Histamine, Bradykinin, Prostaglandin $F_{2\alpha}$, Prostaglandin E_1 , Norepinephrine, Epinephrine, Acetylcholine, Angiotensin	90	385	Umbilical		Contraction	Altura et al. 1972
Histamine, Serotonin		5	Umbilical		Contraction	Dyer et al. 1972
Bradykinin, Epinephrine, Serotonin	87		Umbilical		Contraction	Eltherington et al. 1968
Epinephrine			Umbilical		Contraction	Sparks 1964
Histamine, Serotonin, Norepinephrine			Umbilical		Contraction	Takenaka 1963
Norepinephrine, Epinephrine	1		Ventral branch of left coronary artery		Contraction	Andersson et al. 1972
Norepinephrine, Angiotensin Isoproterenol		7 6	Uterine		Contraction Relaxation	Gough & Dyer 1971
Norepinephrine	16		Lateral epigastric		Contraction	Thulesius & Gjöres 1976
Norepinephrine	22		Mesenteric	1-3	Contraction	Mikkelsen et al. 1979 b, c
5-hydroxytryptamine, Norepinephrine, Histamine Methysergide	12		Extracranial and intracranial arteries		Contraction ----- Inhibition of contraction	Hardeboe et al. 1978

SUBSTANCES	n ₁	n ₂	Type of arteries	Diameter of vessel (mm)	RESPONSE	REFERENCE
5-hydroxytryptamine Norepinephrine Propranolol	8		Basilar		Contraction Reversal of contraction	Boullin & Mohan 1977
Norepinephrine		6	Uterine, splenic, and ileocolic digital		Contraction Contraction	Jauernig et al. 1978
5-hydroxytryptamine Norepinephrine		36 11 14	Basilar temporal femoral		Contraction	Müller-Schweinitzer & Weidmann 1977
5-hydroxytryptamine Norepinephrine Prostaglandin F ₂ α Histamine			Basilar		Contraction	Boullin et al. 1978
Norepinephrine	6 (normo- tensive) 5 (hyper- tensive)		Arteries from the fascia of m. tibialis anterior	0.4	Contraction	Eitinger et al. 1970
Acetylcholine Bradykinin Angiotensin Norepinephrine 5-hydroxytryptamine		11	Umbilical		Contraction	Lewis 1968

SUBSTANCES	n ₁	n ₂	Type of arteries	Diameter of vessel (mm)	RESPONSE	REFERENCE
Norepinephrine	7		"peripheral arteries"	1-4	Contraction	Mikkelsen et al. 1978 b
Acetylcholine		26			12 contraction 8 dilation 6 no response	
Atropine					blocking contraction and dilation	
Epinephrine		32			17 contraction 7 dilation 8 no response	
Norepinephrine		23	Umbilical		13 contraction 4 dilation 6 no response	Gokhale et al. 1966
Isoproterenol		5			no response	
5-hydroxytryptamine					contraction	
Histamine		12			contraction	
Mepyramine		6			blocking contraction	
Bradykinin		9			contraction	
Angiotensin		5			no response	
Aminophylline		8			dilation	

SUBSTANCES	n ₁	n ₂	Type of arteries	Diameter of vessel (mm)	RESPONSE	REFERENCE
Norepinephrine	8 (normo- tensive) 10 (hyper- tensive)		Temporal		Contraction	Horwitz et al. 1974
Norepinephrine 5-hydroxytryptamine			Temporal		Contraction	Glover et al. 1973
Histamine					Reversal of contraction	
Norepinephrine Epinephrine Isoproterenol			Coronary		Relaxing of contracted vessels	Bohr 1967
Norepinephrine			Gastroepiploic, splenic and left ovarian		Contraction	Birmingham et al. 1969
5-hydroxytryptamine		6			6 contraction	Allen et al. 1976
Epinephrine		4			3 contraction	
Norepinephrine		5	Basilar and anterior cerebral		4 contraction	
Acetylcholine		2			1 contraction	
Isoproterenol	6	2			0 contraction	
Bradykinin		2			0 contraction	
Prostaglandin F ₂ α		5			5 contraction	
Prostaglandin E ₁		3			3 contraction	

SUBSTANCES	n ₁	n ₂	Type of arteries	Diameter of vessel (mm)	RESPONSE	REFERENCE
5-hydroxytryptamine Epinephrine Norepinephrine			Basilar, middle and posterior cerebral		Contraction	Shibata & Cheng 1975
Isoproterenol					Relaxation and contraction	
Carbachol					No reaction	
5-hydroxytryptamine Norepinephrine Prostaglandin F ₂ α Prostaglandin E ₁			Basilar		Contraction	Boulin et al. 1976
5-hydroxytryptamine Norepinephrine Histamine	44		Basilar		Contraction	Starling et al. 1975
Mepyramine maleate					blocked histamine contraction	
Histamine Epinephrine Norepinephrine			Common carotid, middle, meningeal, basilar, middle and anterior cerebral		Contraction	Pichler et al. 1953
Histamine Epinephrine			external and internal carotid, superficial temporal		Contraction	
Norepinephrine					No reaction	
Histamine Epinephrine			vertebral, frontal		No reaction	
Norepinephrine			Umbilical, branch of femoral, gastric		Contraction	Holmberg et al. 1976

SUBSTANCES	n ₁	n ₂	Tissue obtained	Diameter of vessels (mm)	RESPONSE	REFERENCE
Acetylcholine		17	at thoracic surgery	"Large" and "small" pulmonary artery branches	C (6), R (7), O (4)	Houghton & Phillips 1973
Norepinephrine		50			C	
Epinephrine		2			C	
Phenylepinephrine		2			C	
Isoproterenol		22			C (4), R (13) O (3), biphasic 2	
Histamine		15			C (13)	
5-hydroxytryptamine		10			C	
Nicotine		14			C (8)	
Norepinephrine	5	6	at thoracic surgery	3-5	C	Joiner et al. 1975a
Acetylcholine		6			C	
Norepinephrine	6	8	at thoracic surgery	2-3	C	Mikkelsen 1979a
Digoxin		5			C	
Nifedine					R	
Potassium		8			C	
Epinephrine	3	3	post mortem		C	Smith & Coxé 1951
Histamine		3			C	
Prostaglandin F ₂ α	9		at thoracic surgery	2-5	C	Joiner et al. 1975b
Prostaglandin E ₁					C (4), R (1), O (4)	
Norepinephrine					C	

Table 8. Pharmacological in vitro investigations on isolated human pulmonary arteries.

n₁ = number of patientsn₂ = number of specimens tested

C = Contraction, R = Relaxation, O = no reaction

variation in reactivity of the same type of artery in different species (Müller-Schweinitzer & Weidmann 1977). Even the same artery show differences in reactivity depending on which part of the artery is investigated. Shibata and Cheng (1975) thus showed that the caudal end of the human basilar artery had the greatest response for 5-hydroxytryptamine, and the middle part showed a greater response to norepinephrine than the caudal part of the artery. When studying pharmacological effects on isolated arteries it therefore is imperative to study the *same segments* (arteries with equal diameter and morphology), to avoid regional, segmental differences. This was done in the present study.

Despite the obvious superiority of investigating *human* material when applying the results to man, numerous investigations have been done with animal tissues, and relatively few experiments have been performed on human vascular tissues. Table 7 compiles investigations made on isolated human arteries obtained at surgery or necropsy with the test-substances used in the present study. Table 8 lists the previous studies on isolated human pulmonary arteries. When no information on a specific topic is given in the tables, actual information in the reference article is lacking. As will be seen, umbilical vessels are the most frequently studied, probably because of the accessibility to such vessels. This may mirror the difficulty in cooperation between laboratory work and clinical medicine, which we have tried to overcome.

In vitro results in this investigation compared to results from previous in vivo studies in humans (Harris & Heath 1977a), and in vitro studies on isolated lungs or pulmonary vessels in different animals (Aviado 1965,

Su & Bevan 1976), are discussed in detail in the different papers of this thesis.

c) *Contractile reactions*

Contraction of isolated human segmental pulmonary arteries is reported in the present study with norepinephrine, epinephrine, histamine, 5-hydroxytryptamine, prostaglandin $F_{2\alpha}$ and SRS-A.

Norepinephrine can contract isolated human uterine, gastroepiploic, epigastric, ileocolic, mesenteric, splenic, left ovarian, common carotid, extra- and intracranial, digital and femoral arteries, as well as arteries from m. tibialis anterior or "peripheral arteries". Both contraction and relaxation after norepinephrine have been reported in coronary and umbilical arteries (table 7).

Epinephrine is also reported to both contract and relax umbilical and coronary vessels, and to contract extra- and intracerebral arteries (table 7).

Histamine contracts extra- and intracranial, basilar, temporal and umbilical arteries, and the contraction of the isolated basilar and umbilical arteries can be blocked by mepyramine (Gokhale et al. 1966, Starling et al. 1975). This corresponds well with our results in pulmonary arteries, indicating that contraction of smooth vascular muscle is a histamine H_1 -receptor effect.

The postulation of two separate – histaminic and adrenergic-receptor systems for pulmonary vascular smooth muscle is in accordance with Bergofsky (1980), who suggests that both the adrenergic and histaminic receptors appear to be able to function independent of postganglionic autonomic or other nervous innervation.

A contraction of isolated human umbilical, basilar, temporal, femoral and extra- and intracerebral arteries is reported with 5-

hydroxytryptamine (table 7), the contraction of the cerebral arteries was inhibited with methysergide (Hardeboe et al. 1978). These results and those on pulmonary arteries reported by Houghton and Phillips 1973 (table 8) and the present studies seem to justify the assumption of a contractory effect of 5-hydroxytryptamine on serotonergic receptors in smooth vascular muscle of man.

Prostaglandin F_{2α} contracts isolated human basilar, and anterior cerebral (table 7), umbilical (Altura et al. 1972) and pulmonary arteries (Joiner et al. 1975b). These results correlate well with our findings of a potent contractory effect on isolated human pulmonary arteries, indicating a direct effect on human arteries by prostaglandin F_{2α}.

Acetylcholine proved to be a potent contractory substance on isolated human pulmonary arteries (II), a reaction also reported by Joiner et al. (1975a), whereas Houghton and Phillips (1973) showed contraction in 6 specimens, relaxation in 7 and no reaction in 4 specimens (table 8). The different actions of acetylcholine in the latter investigation, however, may be due to the use of pulmonary arteries with greater calibre; no specified information on the arteries investigated was given, both "large" and "small" vessels were included. Acetylcholine induces contraction of human basilar, anterior cerebral and umbilical arteries (Altura et al. 1972, Lewis 1968), but Gokhale et al. (1966) reported contraction or relaxation as well as no response (table 7). The contractory effect in the latter study was blocked by atropine. A contractory effect of acetylcholine seems to occur on isolated human vessels, which contrasts with the commonly accepted view of a dilatatory effect of acetylcholine in vivo (II). *Carbachol* can contract basilar, middle and posterior cerebral arteries, and exhibited a contractory effect equal

to acetylcholine on isolated pulmonary arteries (II). This supports the suggestion of a contractory effect of parasympathomimetics on isolated human vessels.

Angiotensin II contracted human pulmonary arteries, and seems not to have been investigated earlier on isolated human pulmonary arteries. Isolated human umbilical and uterine arteries contract from angiotensin (table 7).

d) Relaxation

As there was no resting tone in the segmental pulmonary arteries, a prerequisite for studies of relaxation was induction of some degree of tone. This had to be identical in all experiments, therefore we chose maximal contraction.

Houghton and Phillips (1973) showed relaxation of isolated human pulmonary arteries by *isoproterenol* in 13 of 22 specimens (60 %), whereas a contraction occurred in 4 experiments (table 8). Data are lacking stating whether the vessels were relaxed or contracted prior to the addition of isoproterenol. It is therefore difficult to evaluate the results. The extent of relaxation was not reported. We were able to demonstrate some relaxation in more than 2/3 of the segmental vessels, but a relaxation of more than 30 per cent was found in only six experiments (IV). This correlates well with the results by Gough and Dyer (1971) who obtained a maximal reduction of norepinephrine-induced contraction on isolated human uterine arteries of thirty per cent with isoproterenol (table 7). Both relaxation and contraction have been reported in basilar, anterior, middle and posterior cerebral arteries (Shibata & Cheng 1975, Allen et al. 1976). In umbilical arteries isoproterenol induced no response (Gokhale et al. 1966), while isolated human coronary arteries were

relaxed (Bohr 1967). It is difficult to evaluate the contractory potency in the study of umbilical arteries because information on the tone of the muscles prior to testing is lacking. A relaxing effect of isoproterenol mediated via beta-receptors is likely in isolated vessels both from the lesser (IV) and the major circulation, but the effect is inconsistent.

Theophylline (aminophylline) relaxed isolated human umbilical arteries (Gokhale et al. 1966), results in accordance with ours on contracted isolated pulmonary arteries (IV).

Prostaglandin E₁ relaxed isolated human pulmonary arteries contracted with prostaglandin F_{2α}. Prostaglandin E₁ did not cause any contraction. Joiner et al. (1975b), however, claimed to induce a slight contraction in four specimens, a slight relaxation was found in another specimen and no tension changes were seen in four human isolated pulmonary artery specimens (table 8). Their experiments seems to have been done with uncontracted vessels, however, which explains their inability to show tension changes. Prostaglandin E₁ contracts human umbilical, basilar and anterior cerebral arteries (table 7).

Bradykinin relaxes isolated human pulmonary arteries, whereas it constricts isolated basilar, anterior cerebral and umbilical arteries in man (table 7).

The discrepancies in results obtained on isolated human pulmonary and umbilical arteries stated above may be due to different morphology of the two types of vessels (Brenner 1935, Spivack 1946, Stallabrass 1963).

e) Degenerative changes of pulmonary arteries

Age may cause changing quality or numbers of receptors. Newman et al. (1979) reported

a progressive maturation of histamine receptors in canine pulmonary arteries with a decrease of the number of H₁- and an increase in H₂-receptors with increasing age. With increasing age structural changes have been reported in human pulmonary arteries obtained at necropsies (Harris et al. 1965, MacKay et al. 1978). A decrease of the compliance of human pulmonary arteries with increasing age was ascribed to an increase in medial collagen content by Harris et al. (1965), whereas MacKay et al. (1978) found a statistically significant steady fall in the collagen content of the media with age in 42 arteries from major arterial branches at the lung hilum in patients aged 7–87 years. The studies by MacKay et al. (1978) and Banks et al. (1978) suggest that lower extensibility of pulmonary arteries in higher ages is due to changes in the elastic tissue at a molecular and lamellar level, as the main changes were found in the elastic phase of the vascular stress/strain curve. The medial thickness of the vessel did not change with increasing age. Varying times between death and necropsy complicate the interpretation of these studies, and no information could be given concerning changes of arterial smooth muscle because of autolysis. The studies were done on major pulmonary arteries, which are "elastic" arteries according to the classification of Brenner (1935) and the results are therefore only partially transferable to smaller segmental arteries. Degenerative changes due to age might, however, be expected even in segmental pulmonary arteries. As only light-microscopical examinations were done, there is no refined information about degenerative changes available in the present study, but responses greater than 95 % confidence intervals of the means were found in the one patient below 30 years of age. No conclusions can be drawn from

this single patient, but it seems possible that greater tension changes might be found in young patients without degenerative changes in their vessels.

f) Endocrine function – inactivation of passing mediators

The endocrine function of the lung consists in production of biologically active substances, "local hormones", such as histamine and prostaglandins (Bakhle 1975, Hyman et al. 1978). The lung is also capable of changing the biological activity of a number of mediators passing the pulmonary circulation (intrinsic activity of the lung), whereas other substances pass the lesser circulation unchanged (Junod 1975). Vasoactive substances are transported, catabolized and possibly synthesized in pulmonary endothelial cells (Junod 1977). Norepinephrine, 5-hydroxytryptamine, acetylcholine, prostaglandins E and F and bradykinin are inactivated to varying extent during the lung passage, whereas histamine, isoproterenol and epinephrine pass unchanged through the lung circulation (Junod 1975, 1977, Tierney 1974, Bakhle 1975). Activation of angiotensin I to the biologically active substance angiotensin II takes place in the lung vasculature (Oparil et al. 1971), and trombocytes captured in the lung in lung embolism or trombosis may release prostaglandins (Hyman et al. 1978) and 5-hydroxytryptamine (Junod 1975). These specific features of the lung circulation mean that *the actual local concentrations in the lung vessels of substances such as those tested in this study remain unknown*. For that reason it seems unreasonable to try to compare the doses used in the present investigations with so-called "normal" values (Even et al. 1975). True physiological and certainly pathophy-

siological local concentrations of mediators are virtually unknown.

g) Hypoxia

Chronic hypoxia due to cardiopulmonary diseases and to high-altitude residence (Rotta et al. 1956, Penãloza et al. 1962, Penãloza et al. 1963) leads to pulmonary hypertension. An initial contraction of the vascular smooth muscles in the lung is followed by histological changes in the form of 1) muscularization of the terminal portions of the pulmonary arterial tree, 2) the development of longitudinal muscle in the intima, and 3) a characteristic sparsity of intimal fibrosis in chronic hypoxic patients (Harris & Heath 1977 b). Lloyd (1968) reported that helical strips of pulmonary arteries did not constrict when pO_2 was lowered, whereas O_2 -depletion of strips of the arteries on which a layer of lung remained, caused significantly greater contractions of these strips when compared with the contractions of lung tissue without arterial wall. He suggested that the hypoxic pulmonary vasoconstriction arose from the effect of oxygen depletion on the lung parenchyma (and interstitial retractile cells?) rather than by a direct effect of hypoxia on vascular smooth muscle. Attempts have been made to connect alveolar hypoxia, effect on lung tissue and increase of vascular tension.

The release of vasoactive transmitters such as 5-hydroxytryptamine (Lauweryns & Cokelaere 1973), norepinephrine and epinephrine (Barer & McCurrie 1969, Porcelli & Bergofsky 1973), angiotensin II (Berkov 1974), histamine (Hauge 1968, Haas & Bergofsky 1972) and prostaglandins (Liljestrand 1967, Piper & Vane 1971, Said et al. 1974) from surrounding hypoxic tissue has been given as the cause for the pulmonary vasoconstriction. However, a clear-cut

connection between these mediators and the vasoconstriction has not been established at the present time (Even et al. 1975). Nevertheless, postulation of a hypoxic vasoconstrictory effect for any of the above-mentioned substances, involves their ability to contract pulmonary vessels. In the present investigations it was possible to demonstrate potent contractory effects for several of the possible mediators, i.e. histamine, prostaglandin $F_{2\alpha}$, norepinephrine and epinephrine, whereas the effects of angiotensin II and 5-hydroxytryptamine were of lesser magnitude. All of these substances, therefore, might be involved in alveolar hypoxic vasoconstriction.

h) Studies in one patient with pulmonary hypertension; clinical implications

The results obtained in the single study of a patient with pulmonary hypertension showed that the pulmonary vessels still reacted to prostaglandins and histamine after years of disease. This indicates that mediators may play a role in hypoxic pulmonary vasoconstriction even in advanced stages of the disease. Jose et al. (1975) reported a patient with pulmonary hypertension who had lost his ability to inactivate prostaglandin $F_{2\alpha}$. Disturbances in the endothelial cell metabolism of lung vasculature may perhaps be involved in hypoxic vasoconstriction. The prostaglandin $F_{2\alpha}$ -induced contraction of the isolated human pulmonary arteries could be reversed by prostaglandin E_1 . It might be worth trying to give prostaglandin E_1 to patients with *pulmonary hypertension*. Olley et al. (1976) have consequently been able to diminish severe pulmonary hypertension in children with congenital heart disease by continuous prostaglandin E - infusions during periods prior to cardiac surgery.

i) Mediators and lung embolism

Both broncho- and vasoconstriction are reported in connection with pulmonary embolism (Simonsson & Svedmyr 1977). The chemical mediators, 5-hydroxytryptamine and prostaglandins, substances which contract isolated human pulmonary arteries, are released from platelets in the pulmonary circulation, and have been accused of inducing the pulmonary vasoconstriction (Daicoff et al. 1968, Hyman 1978) as well as the bronchoconstriction seen in lung embolism.

j) Vasoconstriction and antigen - induced bronchial obstruction

During *acute antigen-induced asthma* there are patchy areas of the lungs which are badly perfused. This may be caused by secondary effects from the airways contraction or by direct vascular effects from diffusion of released mediators from the airways or, rather, from the abundant mast cells around the vessels. The sensitivity of the lung vessels to such mediators may thus be of great importance.

k) Limitations of in vitro results

Great caution is necessary in trying to transfer results obtained on a short segment of a specified blood vessel in vitro to effects on the entire circulation in vivo. The further character of a receptor is probably dependent on many varying environmental factors such as hormonal and ionic state, temperature and state of membrane lipids. Very little is known about how these factors influence the receptors (Nickerson & Kunos 1977). An in vitro method uses a standardized environment (pH, temperature, ionic state, oxygenation), but it should not be forgotten that we are dealing with partially denervated preparations in an unphysiological environment.

The complexity of the hemodynamic properties of the *in vivo* pulmonary circulation and its sensitivity to interference from extrinsic factors makes it imperative to minimize extraneous factors in studying fundamental modes of drug action on the pulmonary circulation. For these reasons, an accurate pharmacological characterization of pulmonary vessels must initially be derived from studies of isolated vessels, later supplemented by investigations of the intact lung in the proper species.

General summary

Spirally cut human pulmonary segmental artery strips taken from patients undergoing thoracic surgery, and tested *in vitro*, proved to be a convenient test object for studies of vasomotor changes due to chemical mediators on the responses on pulmonary arterial smooth muscle as a basis for further studies of pulmonary vessels in clinical situations (asthma, hypoxia, pulmonary hypertension). They offer a direct approach for studies of tension changes in a standardized environment. The strips had no resting "active" tone and never showed rhythmic contractions, which makes them suited for quantitative studies, since the variable influence of tone and rhythmic contractions on the responses can be excluded.

None of the patients showed preoperative signs of pulmonary hypertension, and no statistically significant difference could be demonstrated for contractions induced with prostaglandin $F_{2\alpha}$ and histamine between specimens taken from smokers, non-smokers and ex-smokers or from obstructive and normal subjects. Significant differences between effects of different agents used for premedication, muscle relaxation or general anaesthesia could not be demonstrated.

Helically-cut arterial strips were mounted

vertically in a double-walled organ-bath containing oxygenated Krebs solution at $+37^{\circ}\text{C}$. Drugs were administered with micropipettes and compared in $\log \text{ mol/l}$. Contractile tension changes are stated as maximal response induced by supramaximal doses of the agonist in N/m^2 . Contractile responses were reproducible for at least 6–8 hours in the organ-bath at $+37^{\circ}\text{C}$ and for 24–30 hours after storage at $+4^{\circ}\text{C}$. Cumulative dose-response contraction curves over wide concentration ranges were elicited with histamine, prostaglandin $F_{2\alpha}$, norepinephrine, acetylcholine, carbachol, 5-hydroxytryptamine, SRS-A and angiotensin II. Epinephrine showed a dual action with an initial contraction at lower doses followed by a total relaxation at higher doses. The mean maximal contraction induced with histamine was significantly greater than that from all other substances tested. The contractions induced with acetylcholine, prostaglandin $F_{2\alpha}$ and epinephrine were significantly stronger than those induced with 5-hydroxytryptamine or angiotensin II. No statistically significant difference could be observed between the contractions elicited with norepinephrine and epinephrine or between carbachol and acetylcholine, and no significant difference for threshold doses or

ED 50's were found between histamine, prostaglandin $F_{2\alpha}$, acetylcholine or carbachol.

Since the isolated pulmonary arteries had no resting tone, relaxation had to be investigated after an induced increase of tone with contractory substances. The degree of relaxation was calculated in per cent of the total return of the tension to the base-line after a maximal contraction. Relaxation was induced with isoproterenol in 14 of 20 specimens with a mean relaxation ($\pm$ S.E.M.) of 22 ± 5.8 per cent, and with theophylline in 8 of 10 specimens with a mean relaxation of 37 ± 25 per cent. No statistically significant difference was found between the relaxing potency of the two drugs. No relaxation could be induced with the selective β_2 -stimulants salbutamol or terbutaline, or with acetylcholine or carbachol. Prostaglandin E_1 showed a total relaxation in 11 of 21 specimens of prostaglandin $F_{2\alpha}$ -contracted vessels with a mean of 85.5 ± 3.8 per cent. In no experiment was a relaxation less than 55 per cent obtained. A slight relaxation was induced by bradykinin in 18 of 23 specimens; it never exceeded 43 per cent. The relaxation induced with bradykinin on prostaglandin $F_{2\alpha}$ -contracted vessels was 19.4 ± 6.5 per cent which was significantly less than relaxation induced with prostaglandin E_1 ($p < 0.001$).

With the aid of specific antagonists it was possible in the isolated human pulmonary

arteries to postulate the presence of adrenergic α - and β_1 -receptors, muscarinic parasympathetic receptors, serotonergic receptors, histamine H_1 - and H_2 -receptors and angiotensin II-receptors. The presence of receptors for prostaglandin $F_{2\alpha}$ and E_1 is suggested.

Prostaglandin $F_{2\alpha}$ and histamine contracted and prostaglandin E_1 relaxed pathological pulmonary segmental artery specimens taken at autopsy from one patient with primary pulmonary hypertension, indicating that vasomotor changes due to chemical mediators may appear even in morphologically changed vessels. Additional studies of pulmonary vessels from patients with pulmonary hypertension with the described technique are desirable. A comparison of the reactivity of smooth vascular muscle in normals and in patients with pathologically changed pulmonary vessels might produce information valuable for the treatment of e.g. pulmonary hypertension. The difficulties of obtaining specimens from patients with disease in their pulmonary vasculature are obvious; postmortem specimens may well be tested instead.

It is important to test specimens from humans in order to try to infer results to pathophysiological conditions in patients. Results obtained from in vitro studies can form a better basis for extended in vivo studies in human health and disease.

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